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Excretion of Tumor-Associated Antigen(s) in the Urine of Patients With Colon Carcinoma

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Urine samples collected from patients with colon carcinoma and from normal donors were tested for antigenic activity by the microcomplement-fixation assay. When autologous serum was used as the antibody source, 65.4% (17/26) of the urine samples from patients with colon carcinoma were positive for antigen as opposed to only 10% (2/20) from normal volunteers. Absorption of a representative serum with cultured colon cancer cells completely removed reactivity against its autologous urine. Using this serum to screen urine from colon carcinoma patients, antigenic activity was found in 71.4% (30/46) of the samples; however, only 10% (2/20) of the urine samples from apparently healthy volunteers were positive. Analysis of urine samples collected from three patients before and after resection of their primary colon carcinoma and from nine patients undergoing hyperthermia for liver metastases revealed that two of the patients who had curative surgical procedures had marked decreases in urinary antigen levels by the second postoperative day, while the third patient whose disease was unresectable had no significant decrease. Seven of nine patients with metastatic disease had a greater than fourfold increase in antigen activity after hyperthermia and chemotherapy. These results suggest that tumor-associated antigens were excreted into urine, possibly the result of treatment-caused tumor necrosis. Therefore, assessment of tumor-associated antigen(s) in the urine of patients with colon carcinoma may serve as a marker for response to treatment of this disease.

KEY WORDS: urinary antigen, colon carcinoma, complement-fixation, tumor-associated antigen

INTRODUCTION

Tumor-associated antigens have been found both in the kidney glomerulus and the urine of patients with colon carcinoma. In 1974, Couser et al described a patient with colon carcinoma who had nephrotic syndrome and deposits of tumor antigens on the glomerular basement membrane [1]. A novel glycoprotein has been detected in the urine of 64% of patients with metastatic colon carcinoma using rabbit antiserum and double immunodiffusion [2].

Using the microcomplement-fixation assay with autologous or allogeneic serum samples as the antibody source, our laboratory reported finding tumor-associated antigens in the urine of patients with sarcoma and melanoma [3–7]. Correlation of results with the clinical course of these patients suggested that the presence of urinary antigens might be used as a prognosticator for recurrence of the disease. Urinary antigens could not be detected when tumor burden was reduced to very low levels by surgical resection of all gross disease, but reappeared in most patients several months before recurrence

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of the disease was clinically apparent [5]. In view of these findings, this investigation was undertaken to ascertain whether antigens, detectable by autologous and allogeneic antibody, in urine of patients with colon carcinoma could serve a similar role.

MATERIALS AND METHODS

Patients

Urine samples were collected from 42 patients with colon carcinoma, 14 patients with melanoma, and 10 patients with lung carcinoma who were receiving treatment from the UCLA Division of Surgical Oncology (Table I). All patients had gross disease present at the time of urine collection, either as metastatic deposits or as primary tumor before its resection. Urine samples were also collected from 20 healthy volunteers.

Urine Samples

Twenty-four-hour urine samples were collected from all patients. Nine colon carcinoma patients receiving a five-day course of localized hyperthermia and intra-arterial 5-Fluorouracil (5-FU) chemotherapy infusion (thermochemotherapy) for liver metastases had six-hour urine collections performed before and after each daily treatment. Three patients who had laparatomy for primary colon carcinoma had 24 urine collections the day before surgery and for two days postoperatively. Sodium azide (0.5% wt/vol) was added to all samples to prevent bacterial growth during storage at 4°C. Storage was consistently less than 24 hours.

Processing of Urine Samples

Urine samples were centrifuged at 14,000g for ten minutes in a refrigerated centrifuge (Sorvall, CT), filtered through a Whatman #1 filter paper (Whatman, Ltd., England), and concentrated 100-fold using a hollow fiber (10,000 MW exclusion limit) concentrator (Amicon, Lexington, MA). The concentrate was dialyzed against 4 L of 0.025 M phosphate buffer (pH 7.2) supplemented with 0.15 M NaCl and 0.05% sodium azide (PBS). The dialysis was continued for 18 hours with two changes (2 L each) of the buffer. The dialyzed urine was stored at −35°C until used.

TABLE I. Distribution of Cancer Patients and Normal Volunteers Included in This Study With Respect to Sex and Age

Group	No.	Male	Female	Age	
				Range	Mean
Colon carcinoma	42	30	12	43-87	58.2
Lung carcinoma	10	8	2	43-67	58.2
Melanoma	14	7	7	29-71	53.9
Normal volunteers (control)	20	6	14	21-71	37.2
Total	86	51	35	21-87	52.6

Sera

Serum samples were obtained from 26 colon carcinoma patients and 20 healthy volunteers. Samples were stored in liquid nitrogen and decomplemented by heating at 56°C for 30 minutes just prior to use. All sera were diluted 1:8 with veronal-buffered saline (Oxoid Ltd., London, England) before testing against autologous urine samples. A high-reacting serum from a colon carcinoma patient (D.R.) was used as an allogeneic source of antibody (1:8 dilution) to screen all urine samples.

Complement-Fixation Assay

The microcomplement-fixation assay has been described [4]. Briefly, all wells of Terasaki tissue culture plates (Falcon Plastics, Oxnard, CA) were filled with mineral oil of light density. Two μl of increasing doubling dilutions of urine were then placed in the wells and reacted against 2 μl of 1:8 dilution of either autologous or allogeneic serum. Two μl of complement (human umbilical cord serum) representing two units were then added to all wells. After plates were mixed on a Micromixture shaking platform (Cooke Laboratory Products, Alexandria, VA) for 30 seconds and incubated at 37°C for one hour, 2 μl of 0.5% sensitized sheep red blood cells (Flow Labs, Rockville, MD) were added as the indicator system. Following another 30 minutes of incubation at 37°C, the plates were read over a view light (thermolyne, Dubuque, IA). Each plate had controls for complement, buffer, sheep red blood cells, and serum and urine anticomplement activity. The urine titer was defined as the highest dilution of urine that reduced hemolysis of the sheep red blood cells to 50% or less. A urine sample was considered to be positive when its reactivity against an antiserum was at least fourfold above the anticomplementary activity of the urine alone. Using autologous serum, a urine was considered positive if it reacted against antiserum at greater than or equal to 1:8 dilution. With allogeneic serum, a urine sample was considered positive if it reacted at a dilution of 1:16 or greater.

Absorption

Serum (D.R.) was diluted 1:2 with veronal-buffered saline and mixed with an equal volume of packed cells of a colon carcinoma cell line, HT29, and normal liver cells. The mixtures were incubated at 37°C for 30 minutes and centrifuged at 7,000g for ten minutes. After saving an aliquot, supernatants were absorbed once again in a similar manner. The supernatants from first and second absorptions were tested for antibody levels using autologous urine as the target antigens.

Statistical Analyses

The statistical significance of reactivity of urine samples between various groups was determined by the Student's t-test (unpaired) and Fisher's exact test (two-tailed).

RESULTS

Reactivity of Urine With Autologous Serum

Initially, urine samples collected from patients with colon carcinoma and healthy volunteers were reacted against autologous serum samples as the source of antibody. The levels of reactivity of these samples are shown in Figure 1. Sixty-five percent (17/26) of the urine samples from patients with colon carcinoma had antigen titers of 1:8 or greater, whereas only 10% (2/20) of the urine samples from normal volunteers had this level of reactivity ($P < 0.05$). The range (< 1:4-1:256 vs < 1:4-16) and mean ± SE titers (1:33.2 ± 10.3 vs 1:2.9 ± 0.9) were significantly greater for the group of colon carcinoma patients ($P < 0.05$ by Student's t-test).

Antigen Detected With Allogeneic Serum

Serum from one patient (D.R.) who had high antibody activity against his own urine (titer 1:256) was selected as an allogeneic source of antibody to screen all of the urine samples included in this study. Thirty of 42 (71.4%) patients with colon carcinoma had positive urine samples, while only 2/20 (10%) urines from controls had any detectable antigen(s) ($P < 0.05$) (Table II). Mean ± SE antigen titers for both groups (1:75.0 ± 16.6 for colon carcinoma and 1:5.2 ± 1.2 for normal) were higher with allogeneic serum than with autologous serum as the antibody source ($P < 0.05$). Five of 14 (35.7%) patients with melanoma and 2/10 (20%) patients with lung carcinoma also had antigenic activity in urine (mean titers of 1:36.0 ± 18.3 and 1:17.4 ± 12.3, respectively) when tested with D.R.'s serum. Though the mean ± SE titers of positive urine samples of these two groups are not significantly different from those of the colon carcinoma group, the incidence of positive urine samples in the melanoma and lung cancer groups is significantly lower than in the colon group ($P < 0.05$). Figure 2 shows the range of titers for these four groups of patients.

Changes in Antigen Levels Following Surgery

Since the reactivity of urine samples from colon carcinoma patients was significantly higher than healthy volunteers, we analyzed urine samples to determine the effect of surgery. Of three patients with primary colon carcinoma who were positive for urinary antigen(s) preoperatively (titers 1:16, 1:32, and 1:64), two patients

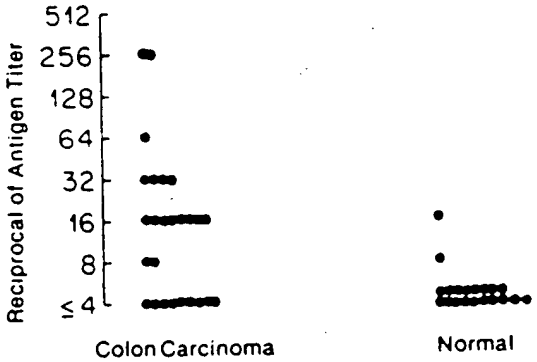

Fig. 1. Distribution of levels of antigenic activity in urine samples from patients with colon carcinoma and normal volunteers against autologous serum samples as the source of antibody. Urine with a titer ≥ 8 considered positive.

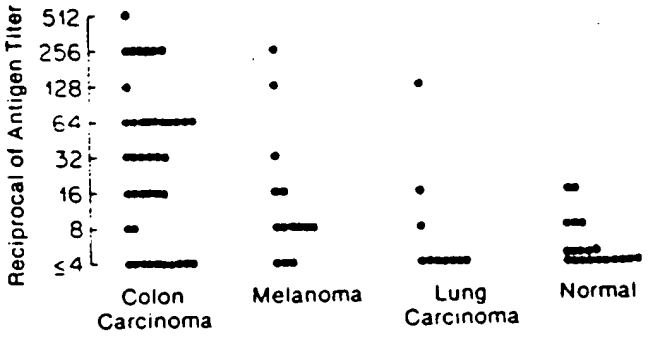

Fig. 2. Distribution of levels of antigenic activity in urine samples from cancer patients and normal volunteers against an allogeneic serum sample (D.R.) as the source of antibody. Urine with a titer ≥ 16 considered positive.

TABLE II. Incidence of Reactivity of Urine Samples Against Allogeneic Serum by Complement Fixation

Group	No. tested	No. positive	Percent positive	Range of antigen titer (reciprocal)	Mean ± SE titer (reciprocal)
Colon carcinoma	42	30	71.4	< 4-512	75.0 ± 16.6
Melanoma	14	5	35.7	< 4-256	36.0 ± 18.3
Lung carcinoma	10	2	20.0	< 4-128	17.4 ± 12.3
Normal volunteers (controls)	20	2	10.0	< 4-16	5.2 ± 1.2

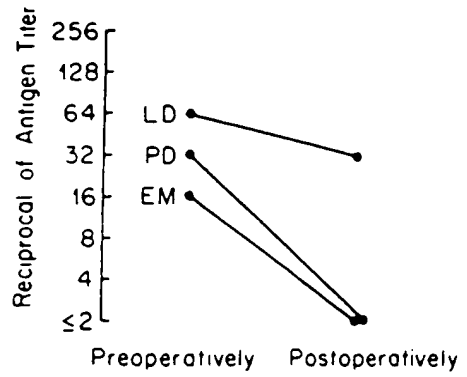

Fig. 3. Levels of antigenic activity against allogeneic serum (D.R.) in pre- and postoperative urine samples of three colon carcinoma patients (L.D., P.D., and E.M.). Complete resection of tumor was possible in patients E.M. and P.D.; however, the tumor was unresectable in patient L.D.

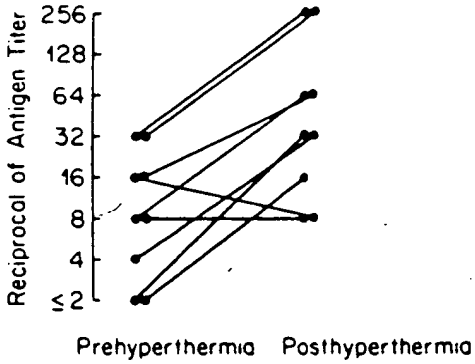

Fig. 4. Effect of thermochemotherapy on urinary antigen levels in nine patients with colon carcinoma.

(E.M. and P.D.) became antigen-negative by the second postoperative day. These two patients had complete resection of their primary tumors (Fig. 3). The third patient, L.D., had an unresectable tumor, and the postoperative urine samples from this patient did not decrease significantly (1:64 – 1:32). This finding suggests that the surgical procedures as such were not responsible for the disappearance of antigenic activity in urine; rather, resection of the tumor was the cause of such a change.

Changes in Antigen Levels Following Hyperthermia and Intraarterial 5-Fluorouracil

It was anticipated that any treatment that caused tumor cell death would increase urinary antigen levels. Since regional thermochemotherapy is one of the protocols applied for the management of patients with colon carcinoma metastatic to the liver, we analyzed urine samples from patients on this protocol for changes in urinary antigen levels.

Of the nine patients who had six-hour urine samples collected during their course of hyperthermia and intraarterial 5-FU treatments, four (44.4%) had positive urines before the first day of treatment (mean titer 1:13.3 ± 3.9 (Fig. 4). However, following only one treatment, 7/9 (77.7%) became positive (mean titer 1:80.0 ± 34.0). All seven patients had significant elevations (greater than or equal to fourfold elevation) in urinary antigen levels with this therapy, while one patient had no change (1:8 – 1:8) and another had a drop in titer (1:16 – 1:8). The tumors of two patients whose urinary antigen levels did not increase after the hyperthermia and intraarterial infusion of 5-FU may be insensitive to the treatments or may not express antigens reactive with the antibodies present in the allogeneic serum.

Absorption Studies

The antigen(s) detected in the urine of patients with colon carcinoma appeared to be tumor-associated. This inference was based on the absorption studies. One absorption of the allogeneic serum (D.R.) with the HT29 (colon carcinoma) cell line resulted in a marked decrease in serum reactivity against D.R.'s urine (antibody titer 1:64 – 1:4) (Table III), whereas two absorptions of the same serum sample with normal liver cells did not decrease serum reactivity (1:64 – 1:32). These results suggest that the antigenic components detected in the urine of patients with colon carcinoma were also expressed by cultured colon carcinoma cells (HT29) but not by normal human liver cells.

DISCUSSION

The results of this study suggest that patients with colon carcinoma excrete antigen(s) into their urine, which is generally not found in the urine of patients without malignancy. Seventeen of 26 (65.4%) patients with colon carcinoma demonstrated autologous reactivity between serum and urine, while only 2/20 (10%) controls had such reactivity. Further screening of urine samples from colon carcinoma patients, controls, and patients with other malignancies (lung carcinoma and melanoma)

TABLE III. Effect on Antibody Activity of Absorption of Allogenic Serum (D.R.) With Colon Carcinoma and Normal Liver Cells

Treatment	Number of absorptions	Reciprocal of serum antibody titer[a]
Unabsorbed	0	64
Normal liver cells	2	32
HT-29 (colon carcinoma) cells	1	4

[a]Autologous urine (D.R.) diluted 1:32 was used as target antigen.

against a high-titer allogeneic serum from a colon carcinoma patient (D.R.) revealed antigenic activity in 30/42 (71.4%) colon carcinoma patients, 2/20 (10%) controls, 5/14 (35.7%) melanoma patients, and 2/10 (20%) lung carcinoma patients. Whether the antigen(s) detected in these groups of patients is the same or different remains to be determined. However, absorption studies performed with the allogeneic serum (D.R.) and a colon carcinoma cell line (HT29) significantly removed serum activity with its autologous urine. Absorption with normal liver cells resulted in no significant decrease in serum antibody, suggesting that the antigen(s) detected was tumor-associated.

Chawla et al reported the presence of a glycoprotein (molecular weight 51,000-59,000) in the urine of 9/14 (64%) colon carcinoma patients using a specific rabbit antiserum and double immunodiffusion and immunoelectrophoresis [2]. This glycoprotein could not be detected in any of 29 control patients but was found in the urine of 15-50% of patients with various other advanced malignancies. Tumor antigens have also been demonstrated in the kidney glomerulus. Couser et al studied a patient with colon carcinoma and the nephrotic syndrome who had a serum antibody that reacted with an antigen deposited on the glomerular basement membrane [1]. Absorption of this serum with homogenates of the patient's tumor abolished all reactivity, suggesting that the antigen was tumor-associated.

Our laboratory has reported the presence of tumor-associated antigens in the urine of patients with sarcoma and melanoma and has demonstrated their clinical usefulness [3-7]. Sequential analysis of urinary antigen titers in sarcoma patients revealed a 4- to 128-fold increase in antigen levels immediately following chemotherapy and radiation therapy. These findings suggest that tumor necrosis had taken place and that tumor antigens had been released into the general circulation and finally into the urine. All patients with an elevated urinary antigen titer preoperatively had a decrease in titer to insignificant levels after excision of all gross tumor. Patients who were followed and subsequently had disease recurrence also had return of urinary antigenic activity 2-9 months before clinical recurrence. Analysis of urine from a group of postoperative stage II melanoma patients revealed a similar rise in antigen levels before disease recurrence. Our study suggests that urinary antigen(s) may be useful for following response to therapy for colon carcinoma. Two of our patients who had complete resection of their primary tumor had decreases in their urinary antigen titer to undetectable levels within 48 hours of operation, while a third patient who had an unresectable tumor had no significant change in titer. Seven of nine patients undergoing local hyperthermia and intraarterial 5-FU for liver metastases had significant elevation in antigen titer, again suggesting tumor necrosis and release of antigens into the circulation. Thus, urinary antigen(s) may be useful not only for detection of the absence or presence of tumor but also to determine the tumor responsiveness (ie, tumor necrosis) to chemotherapy and other tumoricidal forms of treatment.

Though the antigen detected in the urine of patients with colon carcinoma has not yet been fully characterized, our preliminary studies suggest that it has a molecular weight of $>100,000$ daltons and that it is stable at high temperatures (100°C for 15 minutes). The means by which a compound of this high molecular weight passes through the glomerular basement membrane is unknown. There are two possible explanations: First, the urine processing method may cause aggregation of lower molecular weight antigens which initially passed through the glomerulus without difficulty. Second, patients with malignancy may have glomerular damage as a result of immune complex deposition [8-10]. The association of the nephrotic syndrome with malignancy was first reported by Galloway in 1922 [9]. In 1966, Lee et al reported that the nephrotic syndrome developed in over 10% of 101 patients with malignant tumors [10]. However, the exact incidence of subclinical immune complex nephritis is unknown and may be present in a large percentage of patients with malignancy. Thus, deposition of antibody-tumor antigen complexes on the glomerular basement membrane as described by Couser et al [1] may alter the permeability of the membrane and allow large molecular weight compounds to pass into the urine.

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REFERENCES

1. Couser WG, Wagonfeld JB, Spargo BH, Lewis ET: Glomerular deposition of tumor antigen in membraneous nephropathy associated with colon carcinoma. Am J Med 57:962-970, 1974.
2. Chawla RK, Heymsfield SB, Wadsworth AA, et al: Isolation and characterization of glycoprotein (IBBS) in the urine of a patient with carcinoma of the colon. Cancer Res 37:873-878, 1977.
3. Gupta RK, Morton DL: Detection of cancer-associated antigen(s) in urine of sarcoma patients. J Surg Oncol 11:65-74, 1979.
4. Gupta RK, Morton DL: Presence of human tumor-associated antigen(s) in urine of patients with cancer. Surg Forum 26:158, 1975.
5. Huth JF, Gupta RK, Morton DL: Sequential analysis of urinary antigen(s) in patients with sarcoma. Surg Forum 30:150-152, 1979.

6. Rote NS, Gupta RK, Morton DL: Determination of incidence and partial characterization of tumor-associated antigens found in the urine of patients bearing solid tumors. Int J Cancer 26:203-210, 1980.
7. Rote NS, Gupta RK, Morton DL: Tumor-associated antigens detected by autologous sera in urine of patients with solid neoplasms. J Surg Res 29:18-22, 1980.
8. Gagliano RG, Castanzi JJ, Beathard GA, et al: The nephrotic syndrome associated with neoplasia: An unusual paraneoplastic syndrome: Report of a case and review of the literature. Am J Med 60:1026-1031, 1976.
9. Galloway J: Remarks on Hodgkin's disease. Br J Med 2:120, 1922.
10. Lee JC, Yamanchi H, Happer J Jr: The association of cancer and the nephrotic syndrome. Am Int Med 64:41-51, 1966.